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ORIGINAL COMMUNICATION

Uric acid levels in sera from patients with multiple sclerosis

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Abstract The levels of uric acid (UA), a natural peroxynitrite scavenger, were measured in sera from 240 patients with multiple sclerosis (MS) and 104 sex- and age-matched control patients with other neurological diseases (OND). The mean serum UA concentration was lower in the MS than in the OND group, but the difference did not reach the level of statistical significance (P=0.068). However, the mean serum UA level from patients with active MS (202.6+67.1 μmol/l) was significantly lower than that in inactive MS patients (226.5+78.6 μmol/l; P=0.046) and OND controls (P=0.007). We found a significant inverse correlation of serum UA concentration with female gender (P=0.0001), disease activity (P=0.012) and duration (P=0.017), and a trend towards an inverse correlation with disability as assessed by EDSS score, which did not reach statistical significance (P=0.067). Finally, multivariate linear regression analyses showed that UA concentration was independently correlated with gender (P=0.0001), disease activity (P=0.014) and duration of the disease (P=0.043) in MS patients. These findings suggest that serum UA might serve as a possible marker of disease activity in MS. They also provide support to the potential beneficial therapeutic effect of radical-scavenging substances in MS.

Key words Multiple sclerosis · Uric acid · Peroxynitrite · Disease activity · Magnetic resonance imaging

Introduction

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) in which an autoimmune pathogenetic mechanism has been postulated. It is proposed that soluble products generated from infiltrating immune cells and glial cells contribute to the damage to myelin and oligodendroglia in MS [19]. Among these, nitric oxide (NO) has been implicated in MS and its animal model, experimental autoimmune encephalomyelitis (EAE). The levels of reactive oxygen and nitrogen species can increase dramatically under conditions such as inflammation, and this can overwhelm the inherent antioxidant defences within lesions. Damage to DNA found in MS lesions and in neuron-rich areas located in the

proximity of these lesions is attributed to the release of reactive oxygen species and NO during inflammation in the brain [26]. When NO and superoxide are formed simultaneously, they may react to form the powerful oxidant peroxynitrite ($ONOO^-$) at a rate constant which is three times faster than that at which superoxide dismutase scavenges superoxide [3].

It is now believed that many of the deleterious effects previously ascribed to NO may be in fact due to peroxynitrite. Peroxynitrite can damage subcellular organelles, membranes and enzymes through its action on proteins, lipids and DNA, including the nitration of tyrosine residues of proteins [3]. Peroxynitrite may also act through its metabolites, nitrogen dioxide and hydroxyl radical, and through toxic oxidant species [12]. Nitrotyrosine, which serves as a biochemical marker for peroxynitrite-mediated damage, has been detected immunohistochemically within CNS tissues of MS patients and in most of the MS sections displaying inflammation [7]. Extensive peroxynitrite activity has also been identified during early stages of EAE [23]. Peroxynitrite-induced myelin oxidation may represent an early process in EAE development. Indeed, it has recently been shown that EAE can be prevented by peroxynitrite scavengers [9, 10, 11]. Uric acid (UA), which is the final product of the common pathway of purine nucleoside metabolism, is a strong peroxynitrite scavenger. The administration of UA to treat EAE in mice has been shown to produce a strong beneficial effect. Furthermore, it was demonstrated that 46 patients with MS had significantly lower levels of serum UA than controls, and that MS and gout (hyperuricaemia) were virtually self-exclusive [10].

These findings raise questions concerning the role of UA in the pathogenesis and potentially treatment of MS. We therefore compared serum UA levels in patients with MS and in controls with other neurological diseases (OND). We also looked for a possible association between serum UA concentrations and the following clinical and demographic characteristics of MS patients: sex, age, course of the disease, clinical and magnetic resonance imaging (MRI) disease activity, disability as assessed by Expanded Disability Status Scale (EDSS) score [15] and disease duration.

Materials and methods

Blood samples were collected from 240 patients with clinically or laboratory-supported definite MS according to the criteria of Poser et al. [18] who were admitted to the Institute of Neurology, Belgrade over a period of 18 months. There were 66 men and 174 women (M/F 1:2.6). Their mean age was 37.2 ± 11.2 years (median 36.5; range 18–75), mean duration of disease 6.2 ± 6.0 years (range 0.5–37) and the EDSS score 4.4 ± 2.0 (range 0–9.5). Of the 240 patients 154 had the relapsing-remitting (RR) type of the disease, 57 secondary progressive (SP) and 29 primary progressive (PP). Of the 154 RR patients 68 were in exacerbation and 86 in remission. Exacerbation was defined as a sudden appearance of new symptoms and signs or reappearance or worsening of previous findings lasting more than 24 h, occurring within 2 weeks of blood sampling. Patients with progressive MS were considered to have active disease when the EDSS score had increased by at least 1 point in the previous year. Accordingly, 118 patients (68 RR, 32 SP, 18 PP) were judged to have clinically active MS while the remaining 122 (86 RR, 25 SP, 11 PP) had clinically inactive disease. Clinical and demographic characteristics of MS patients, grouped according to the various clinical types, are presented in Table 1.

Six patients with RR MS were followed-up for 4–12 weeks and UA concentrations measured during various phases of clinical activity.

In a subgroup of 25 MS patients, brain magnetic resonance imaging (MRI) with gadopentetate dimeglumine (Gd-DTPA) injection was performed on the same day on which blood was drawn, performed on a Siemens Magnetom (1.0 T). The scanning protocol included T2-weighted spin-echo images (TR=4000 ms, TE=90 ms) and T1-weighted spin-echo images (TR=560, TE=15 ms) in the transverse plane with 5-mm slice thickness. Gd-DTPA was given intravenously at a dose of 0.1 mmol/kg, and about 15 min after contrast injection the T1-weighted sequence was repeated. The same experienced neuroradiologist determined the presence of Gd-DTPA-enhancing lesions.

Serving as controls were a group of 104 age- and sex-matched patients with other neurological disorders (30 epilepsy, 19 migraine, 16 tension headache, 11 lumbar disc herniation, 6 hereditary ataxia, 5 encephalitis, 3 subacute combined degeneration, 3 neurosarcoidosis, 2 neurolupus, 2 antiphospolipid syndrome, 2 cysticercosis cerebri, 2 cerebral contusion, 2 chronic inflammatory demyelinating polyradiculoneuropathy, 1 motor neuron disease).

All MS patients and controls gave their informed consent prior to their inclusion in the study, which was approved by the local ethics committee. Excluded from the study were patients with chronic renal disease and diabetes mellitus and those receiving acetylsalicylic acid, thiazide diuretics, steroids or other drugs which have been reported to affect serum UA levels, at the time of blood sampling [27]. None of the patients suffered from gout. All patients received the same diet in the hospital for 7 days prior to blood sampling, since diet may influence serum uric concentration based on its purine content. Blood samples were drawn from an antecubital vein after overnight fasting and immediately frozen in aliquots at –20°. UA levels in sera were determined by using a commercially available enzymatic, colorimetric assay according to the manufacturer's instructions (Boehringer-Mannheim, Mannheim, Germany). In our institution (Clinical Centre of Serbia, Belgrade, Yugoslavia), the normal range of serum UA values is 140–400 μmol/l in women and 200–460 μmol/l in men.

Tab. 1 Demographic and clinical characteristics of 240 multiple sclerosis (*MS*) patients and 104 patients with other neurological diseases (*OND*) (*RR* relapsing-remitting, *SP* secondary-progressive, *PP* primary-progressive, *EDSS* Expanded Disability Status Scale)

Variables	RRMS	SPMS	PPMS	Total MS	OND controls
Age (years)	34.6 ± 10.3	41.5 ± 12.4	42.6 ± 8.9	37.2 ± 11.2	41.9 ± 12.6
Sex: male/female (ratio)	35/119 (0.29)	17/40 (0.42)	14/15 (0.93)	66/174 (0.38)	30/74 (0.40)
Disease duration (years)	4.9 ± 5.1	10.4 ± 7.9	4.9 ± 3.3	6.2 ± 6.0	–
Active disease	68 (44.2 %)	32 (56.1 %)	18 (62.1 %)	118 (49.2 %)	–
EDSS score	3.3 ± 1.5	6.6 ± 1.5	5.6 ± 1.2	4.4 ± 2.0	–

Student's *t* test and the Wilcoxon/Mann-Whitney *U* test were used for comparisons between groups. The paired-*t* test was used for the statistical evaluation of differences in UA concentrations in patients who were prospectively followed-up. In MS patients the correlations were made between variables using the Pearson linear regression model. In addition, multivariate regression models for MS patients, OND controls and the total study group (MS patients + OND controls) were calculated to detect the variables with an independent effect upon the UA level.

Results

The lowest mean UA level was found in patients with SPMS, this being significantly lower than the level in controls (*P*=0.016) (Table 2). On the other hand, the mean level in PPMS and controls was almost identical (*P*=0.679). In the overall MS group, serum UA levels were lower than in controls, but the difference did not reach statistical significance (*P*=0.068).

The mean serum UA level in the clinically active MS group (202.65±67.14 µmol/l), comprising patients with RR, SP and PP disease, was significantly lower than that in clinically inactive MS patients (226.51±78.57 µmol/l; *P*=0.046) or controls (*P*=0.007; Fig. 1 a). RR patients in relapse and those in the active phase of SPMS had significantly lower values than controls, while no such difference was observed between active PP patients and controls (Table 2).

In the subgroup of 25 MS patients (13 clinically active, 12 clinically inactive) in whom brain MRI with Gd-DTPA injection were performed, one or more Gd-DTPA-enhancing lesions were present in 15 (60 %). The highest mean value of UA was obtained in sera from patients with clinically inactive disease who had no Gd-DTPA enhancing lesions, whereas patients with clinically active disease and those with clinically inactive MS, but with active, Gd-DTPA-enhancing lesions, had lower, almost identical values (Fig. 1 b). The differences between the groups did not reach statistical significance, presumably due to the small number of patients involved.

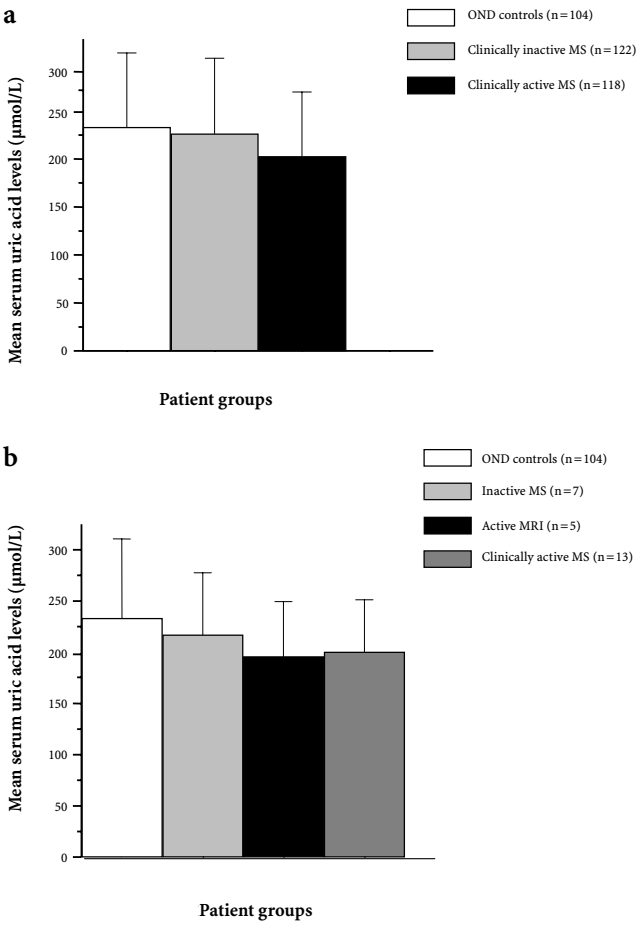

Fig. 1 **a** Mean serum uric acid levels from patients with clinically active MS (*n*=118), clinically inactive MS (*n*=122) and 104 OND controls. A significant difference was observed between clinically active and clinically inactive MS (*P*=0.046) and controls (*P*=0.007). Mean values are shown with standard deviation. **b** Mean serum uric acid levels from patients with clinically active MS (*n*=13), clinically inactive patients with active lesions on brain MRI (*n*=5), clinically inactive patients without active lesions on brain MRI (*n*=7) and 104 OND controls. No significant differences were observed. Mean values are shown with standard deviation

Tab. 2 Serum uric acid concentrations (µmol/l) in 240 patients with multiple sclerosis (*MS*), in relation to different clinical subtypes, and 104 controls with other neurological disorders (*OND*) (*RR* relapsing-remitting, *SP* secondary-progressive, *PP* primary-progressive)

Disease groups	*n*	Mean±SD	Median	Range
Total MS	240	214.8±74.0	205.2	58.0–420.0
RR	154	217.5±73.1	208.0	62.0–417.9
Relapse	68	203.3±67.4*	197.2	62.0–404.7
Remission	86	228.8±75.0	222.0	107.0–417.9
SP	57	200.4±71.2**	187.0	58.0–396.7
Active	32	181.3±57.2***	183.6	58.0–325.0
Inactive	25	218.4±85.6	212.0	110.0–396.7
PP	29	228.5±80.7	209.0	111.9–450.0
Active	18	229.3±76.7	207.5	121.0–357.9
Inactive	11	227.0±89.9	215.0	111.9–420.0
OND controls	104	233.2±77.5	210.9	110.0–445.5

*P=0.026 RRMS in relapse vs. controls, **P=0.016 SPMS vs. controls, ***P=0.007 active SPMS vs. controls

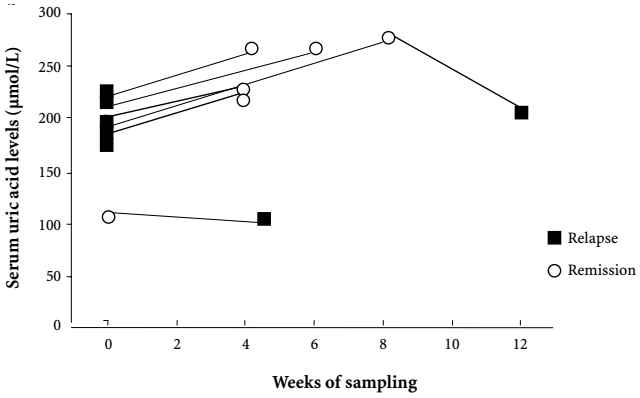

Fig. 2 Repeated measurement analysis of serum uric acid levels in six relapsing-remitting multiple sclerosis patients. Blood samples were obtained during the clinical relapses and remissions. The difference between uric acid levels in relapses and remissions was significant (P=0.006)

The prospective follow-up analysis of UA levels in serum from six patients with RRMS revealed fluctuations during the observation period. In these patients repeated measurement analysis showed a significantly higher mean UA level during remissions (227.41±62.43 μmol/l) than during relapses (182.91±43.02 μmol/l) (P=0.006) (Fig. 2).

We also found a significant inverse correlation of serum UA concentration with female gender (r=–0.429, P=0.0001), disease activity (r=–0.162, P=0.012) and duration (r=–0.154, P=0.017), and a trend towards an inverse correlation with disability as assessed by EDSS score (r=–0.119, P=0.067). There was no correlation between clinical course and serum UA concentrations (r=–0.003, P=0.968).

Multivariate linear regression analyses showed that serum UA is independently correlated with gender (P=0.0001), disease activity (P=0.014) and duration (P=0.043) in MS patients (Table 3). Gender was also correlated with serum UA in controls (P=0.0001). Other variables analysed in the multivariate models (age in MS patients, OND controls and the total study group; EDSS and disease course in MS patients) were not independently correlated with serum UA concentration in any of the study groups. However, it should be emphasized that MS course was correlated with gender (r=–0.178, P=0.006), EDSS (r=0.582, P=0.0001), activity (r=0.136, P=0.035) and duration of the disease (r=0.161, P=0.013), suggesting that the lowest value of UA in patients with SPMS and the highest in PPMS were due to the unequal distribution of these demographic and clinical measures among patients with various subtypes of MS (Table 1).

Tab. 3 Variables hown by multivariate linear regression analysis to be significantly correlated with uric acid levels in patients with multiple sclerosis

Variables analysed	t	β	Standard error	P
Sex	−7.123	−0.412	9.565	0.0001
Disease duration	−2.039	−0.118	0.689	0.043
Disease activity	−2.464	−0.142	8.511	0.014

Discussion

In the present study we observed lower levels of UA in sera from patients with clinically active MS than in sera from clinically inactive MS patients or controls with OND. Additionally, in six RRMS patients in whom we performed repeated serum UA levels analyses we observed an increase in serum UA level during remission and decrease during relapse. We also found lower serum UA concentrations in the overall MS group than in the OND group, but the difference was statistically non-significant.

UA levels in sera from MS patients have been recently reported in two studies [10, 13]. One found serum UA levels to be significantly lower in MS patients than in patients with OND [10], while the other observed no such difference [13]. However, the correlation between UA levels and clinical measures of MS was not tested in these studies. More recently Constantinescu et al. [6] reported elevation in the mean serum UA levels in MS patients after 6 months of treatment with glatiramer acetate, suggesting that beneficial effect of this drug is based on elevation in UA, as a natural scavenger of peroxynitrite.

We found significantly lower values of serum UA in SPMS patients than in controls, while significant differences were observed neither between RR or PPMS and controls nor between the different clinical subtypes of MS. However, after performing multivariate linear regression analyses we concluded that low values of UA in SPMS were not due to the independent effect of SP disease course. Our SPMS patients may have had lower serum UA concentrations due to the relative predominance of female gender, active disease, and longer disease duration in this subgroup of patients since we found strong inverse correlation between these variables and serum UA concentration. Inverse correlation between serum UA concentration and female gender may be one of the factors that influence female predominance in MS.

As noted above, we found significantly lower concentrations of UA in sera from patients with clinically active disease than in those with clinically inactive disease or controls. Moreover, we found an independent effect of disease activity on serum UA in MS patients by the multivariate regression analyses. A trend towards lower values was also observed in patients with Gd-DTPA enhancement on brain MRI as a sign of MRI disease

activity in comparison with those without active lesions, but the difference did not reach statistical significance, perhaps due to the small number of patients in whom brain MRI with Gd-DTPA injection was performed. Since Gd-DTPA enhancement in MRI of the brain in MS is associated with inflammation [4], our results may indicate a significant role for UA, as a peroxynitrite scavenger, in CSF inflammation in MS. Whether the reduction in UA level in patients with active MS is a cause or a consequence of disease activity in MS remains uncertain. It may be speculated that patients with active MS have an intrinsically reduced antioxidant reserve which contributes to the development of CNS inflammation and tissue damage in MS, or that CNS inflammation in the active phase of the disease leads to the consumption of UA as scavenger. In favour of the former, Hooper et al. [11] recently reported that UA protects the integrity of the blood-CNS barrier in mice with EAE such that inflammatory cell migration into CNS tissues is reduced. However, the same study showed that exogenously administered UA penetrates the already compromised blood-CNS barrier and blocks peroxynitrite-mediated tyrosine nitration and apoptotic cell death within the areas of inflammation in spinal cord in EAE, speaking in favour of the latter notion.

Although inflammation and demyelination are central features in MS, recent observations from pathological studies and magnetic resonance spectroscopy have led to the hypothesis that axonal damage is responsible for a significant proportion of the clinical phenomena and irreversible neurological impairment in this disease [5, 22]. Axonal damage, represented by decreases in brain *N*-acetylaspartate concentrations in magnetic resonance spectroscopy studies, was shown to progress over time [2] and to be correlated with clinical disability [1]. The observation from pathological studies that axonal transection is most prominent in areas of active demyelination and inflammation suggests that axonal damage is caused by cytokines or free radicals [8, 22]. Our findings of a significant inverse correlation of serum UA concentration with disease activity and duration and a trend towards the inverse correlation with disability, as assessed by EDSS score (P=0.067), implies that inadequate protection against the activity of peroxynitrite by UA plays a role in axonal loss and progressive CNS tissue damage in MS. T2-weighted MRI is poorly correlated with disability. However, new putative magnetic resonance markers of axonal loss and irreversible tissue damage which include reduced concentrations of *N*-acetylaspartate on magnetic resonance spectroscopy, T1-weighted hypointense ('black holes') total lesion load, decreased magnetization transfer ratio, and brain atrophy, have demonstrated improved correlation with disability [5, 17, 25]. Therefore studies need to be carried out on the correlation between UA and longitudinal changes in these specifically magnetic resonance derived indices and clinical disease progression in order to further elucidate its potential role in preventing tissue damage in MS.

The current evidence suggesting a significant role for peroxynitrite in the pathogenesis of MS is still elusive since it is based mainly on data from in vitro experiments and animal models. It has been confirmed that NO and its toxic metabolite peroxynitrite inhibit components of the mitochondrial respiratory chain leading, if damage is severe enough, to a cellular energy deficiency state [21]. Such oxidative and/or nitrative stress can damage the lipids, proteins and nucleic acids of cells and mitochondria, potentially causing cell death. It has been demonstrated that both NO and peroxynitrite may possibly have a role in the process of demyelination by inducing oligodendrocyte death [16] and through damage of the myelin sheath by inducing lipid peroxidation [24]. Moreover, NO donors have been shown to cause reversible conduction block in both normal and demyelinated axons of the central and peripheral nervous systems [20]. Conduction in demyelinated and early remyelinated axons is particularly sensitive to block by NO, so that at lower concentrations, including those expected at sites of inflammation, demyelinated axons are selectively affected.

Our findings suggesting that the elevation in UA concentration reduces CNS inflammation and tissue damage may have significant therapeutic implications. In accordance with this notion, the beneficial therapeutic effect of UA in acute and chronic form of mouse EAE [9, 10] has been already demonstrated. UA, a natural scavenger of peroxynitrite, inhibits the onset of EAE in an acute, aggressive form of the disease [9] and promotes long-term survival even if therapy began only after the onset of clinical symptoms [10]. Similar beneficial results of treatment with UA have been observed in experimental pneumococcal meningitis [14] and in focal brain injury in rats [28] confirming its potential therapeutic value for diseases whose pathogenesis presumably involves the deleterious effects of peroxynitrite. Therefore the current study also supports the administration of radical-scavenging substances early in the course of the disease potentially to prevent the inflammation and development of irreversible neurological deficit in MS.

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